## **APHIS Evaluation of the African Swine Fever Status of Portugal**

Animal and Plant Health Inspection Service Veterinary Services

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## EVALUATION OF THE AFRICAN SWINE FEVER STATUS OF PORTUGAL

#### Introduction

On November 15, 1999, an outbreak of African Swine Fever (ASF) was confirmed in the region of Alentejo in Portugal [1]. Portugal had previously been recognized free of ASF by USDA-APHIS [2].

On January 18-19, 2001, a team from APHIS conducted a site visit in Portugal to evaluate Portugal's status with respect to ASF [3]. The APHIS team met with officials in Lisbon and visited the laboratory facilities there, and traveled to the region of Alentejo to see the area where the outbreak occurred. Detailed information about the control and eradication of the outbreak was obtained. The primary contact person for the visit was Dr. Antonio Jose Rosinha, Director of Veterinary Services. The team was also accompanied by the following European Commission representatives: Alberto Laddomada, DG-SANCO, Brussels, and Eva Zamora, FVO, Dublin.

In assessing the ASF status of Portugal and in accordance with its regulations [4], APHIS evaluated the following factors based on information obtained from the site visit and other sources. These factors were considered in relation to the 1999 outbreak in Alentejo and historical data provided by veterinary officials in Portugal and other interested parties [5-12].

- Authority, organization, and infrastructure of the veterinary services in the region
- Disease surveillance in the region
- Diagnostic laboratory capabilities
- Disease status of the region
- Active disease control program, if any, if the agent is known to exist in the region
- Vaccination status of the region
- Disease status of adjacent regions
- Separation of the region from regions of higher risk through physical or other barriers
- Control of movements of animals and animal products from regions of higher risk
- Livestock demographics and marketing practices in the region
- Emergency response capability

A summary of the data relating to each of these factors is presented below.

### **Description of the disease** [13, 14].

ASF is a highly contagious and tick borne disease of swine. Hosts of ASF virus include domestic and wild pigs, i.e., the warthog, bush pig, and giant forest hog in Africa and wild boar in Europe. African wild swine are usually inapparently infected. The virus replicates in ticks of the genus *Ornithodoros* and there is transstadial, transovarial, and sexual transmission in these arthropods [3].

In domestic swine, the clinical signs of ASF are dependent upon the virulence of the virus. The highly virulent form of the virus causes acute disease characterized by high fever, reddening of the skin in white pigs, anorexia, cyanosis, listlessness, and incoordination. Mortality approaches 100 percent, and death occurs within 6 to 13 days. Lesions of acute ASF include pronounced hemorrhages in the gastrohepatic and renal lymph nodes; petechial hemorrhages of the renal cortex and also in medulla and pelvis of the kidneys; congestive splenomegaly; excess fluid in the pleural, pericardial and/or peritoneal cavity; petechiae in the mucous membranes of the larynx and bladder; petechiae on the visceral surfaces of organs; and edema in the mesenteric structures of the colon and the wall of the gall bladder.

Moderately virulent ASF virus causes a subacute disease with less intense clinical signs and lesions and a lower mortality rate. In pigs that die, death usually occurs between 15 and 45 days post infection. Low virulence virus also exists which may only cause animals to seroconvert. A chronic form of the disease has been reported which develops over 2-15 months. Various signs have been described including loss of weight, irregular peaks of temperature, respiratory signs, necrosis in areas of the skin, chronic skin ulcers, and arthritis. Pericarditis, adhesions of the lungs, caseous necrosis and mineralization of the lungs, and enlarged lymph nodes may be seen on necropsy of pigs with the chronic form of ASF.

ASF virus is transmitted directly by contact between sick and healthy animals. It is also transmitted indirectly by soft ticks of the genus *Ornithodoros*, by the feeding of garbage containing infected meat, and through fomites, i.e., on premises, vehicles, implements, and clothing. Sources of virus include blood, tissues, secretions and excretions of sick and dead animals. A carrier state exists, especially in African wild swine and in domestic pigs in enzootic areas.

ASF is caused by a large DNA virus that has characteristics of both an iridovirus and a poxvirus. ASF virus is highly resistant to low temperatures. It is heat inactivated at 56°C for 70 minutes or 60°C for 20 minutes. It is also inactivated at a pH <3.9 or >11.5 in serum-free medium. The presence of serum greatly increases the virus' resistance to inactivation at a low or high pH. It remains viable for long periods in blood, feces and tissues.

## The authority, organization, and infrastructure of the veterinary services organization in the region

The official veterinary services of Portugal are organized at several levels. At the national level, the *Direccao Geral de Veterinaria* (DVG), is part of the Ministry of Agriculture, Rural Development and Fisheries. Personnel resources at the DVG level consist of approximately 145 veterinarians out of a total staff of about 450.

At the regional level, the *Direccao de Servicos de Veterinaria* (DSV) form part of the Regional Agriculture Boards. There are seven Regional Agriculture Boards in mainland Portugal and one each in the Azores and Madeira. The DGV provides technical coordination to the DSVs, but the latter units are administratively independent.

The DSVs operate with local units (DIV, *Divisoas de Intervencao Veterinaria*) which in turn are divided into smaller administrative units or municipalities. About 615 veterinarians are employed at the regional and local levels, including at least one official veterinarian in each municipality. The DIVs supervise activities related to animal identification, herd registration, movement permits, disease eradication, and contingency plans. The authority and enforcement powers related to such activities are found in several different national laws [15].

### The type and extent of disease surveillance in the region

Surveillance during the course of the 1999 outbreak is described later in this document. Surveillance for additional cases continues, with samples obtained from abattoirs, wild boars, and routine diagnostic testing. All swine samples submitted for diagnostic analysis are tested for ASF, regardless of the suspected disease. In total, during the year 2000, ASF testing was conducted on 9,726 samples from abattoirs, 46,823 from herds in Alentejo and the adjacent region of Algarve, 1,030 from herds in other regions, 2,278 from wild boars, and 579 in random monitoring of imported pigs. As part of this routine surveillance, testing for other diseases such as classical swine fever, swine vesicular disease, Porcine Reproductive and Respiratory Syndroma, Aujeszky's disease, and foot and mouth disease are also included. No additional evidence of ASF has been found.

The major risk factor identified by APHIS is the possibility of persistence of ASF virus in ticks. The virus was isolated from ticks on the index farm and one of the contact premises, and subsequent surveillance was conducted to determine if infected tick populations existed elsewhere. Tick surveillance is discussed in the section below on Disease Status.

### Diagnostic laboratory capability

Diagnostic testing for ASF is conducted in the Central Veterinary Laboratory in Lisbon, using procedures prescribed by the Office International des Epizooties [16]. The primary serological test used is the ELISA. The laboratory is using its own ELISA test, not one of the commercial kits. The hemadsorption test and direct immunoflourescence are used for virological testing.

As will be described below, surveillance was also conducted in ticks. Ticks were combined into a pool of 5-6 ticks, grouped by life stage and status (engorged versus non-engorged). Tick homogenates were inoculated into buffy coat cultures and the presence of ASF virus was demonstrated by hemadsorption and confirmed by direct immunofluorescence. PCR was also used in the attempt to demonstrate virus in ticks, but has not been completely successful.

The laboratory was experiencing some disruption during the site visit, due to basic repairs. The laboratory is housed in a very old building and a new facility is being constructed. The virology laboratory had been moved to temporary quarters during the repairs, but seemed to be functioning adequately given the disruption. The laboratory also collaborates with the faculty at the veterinary college.

#### Disease status

The outbreak of ASF was confirmed on November 15, 1999. Prior to this outbreak, the disease had not been known to occur since 1993. At that time, Portugal had eradicated the disease. Surveillance had continued in various forms since eradication of ASF in 1993.

On November 5, 1999, a producer in Alentejo reported a suspicion of disease in his pigs. Clinical signs were very mild. Samples were obtained and sent to the laboratory. As part of continuing surveillance for ASF, all porcine diagnostic samples submitted to the laboratory are tested for ASF. Serological results on the ELISA test were positive on November 9. The swine herd was slaughtered on November 11-12, a total of 108 animals, of which 6 were already dead. There were 2 adjacent herds involved and they were considered to be one epidemiological unit due to contact across a single fence line. Virus isolation was positive for ASF on November 15.

A protection zone of 3 km and a surveillance zone of 10 km were established around the infected premises and other actions taken in accordance with European Community requirements [17]. A stamping-out policy was adopted in the protection zone, and approximately 1000 animals were slaughtered in this zone. Clinical inspections and sampling were started in the surveillance zone on November 18, 1999. Each herd in the surveillance zone was sampled twice. One herd in the surveillance zone was positive and was depopulated. It was determined that this herd had contact with the outbreak farm. All pigs in the remainder of the Alentejo region were also sampled (15,000 pigs from 400 farms) with negative results. In addition, surveillance was also done at abattoirs on pigs moving from the restricted zones, again all with negative results. Wild boar surveillance was increased during the 1999 hunting season. A total of 1,781 samples from wild boar were obtained and tested, all with negative results.

There was a total of 4 herds (three in the protection zone, one in the surveillance zone) with antibodies to ASF virus and all four were depopulated. The epidemiological investigation revealed animal and human contact with the outbreak farm. Two herds had positive virus isolation, the index herd and another herd considered part of the same epidemiological unit. These two herds are not included in the above results for antibodies. ASF virus was isolated both from animals and from ticks on these two premises.

Two violations of animal movement controls were noted, one from the surveillance zone and one from the protection zone. In both cases, the pigs and/or carcasses were destroyed and the owner was penalized.

The virus involved in these outbreaks was characterized as being closely related to the Lisbon/60 isolate. This is the virus that caused outbreaks in the southern part of Portugal from 1960 to 1993. The source of the 1999 outbreak was not identified; however, it was hypothesized that the outbreak was associated with viral persistence in ticks [18]. To test this hypothesis, ticks were collected for virus testing, based on a risk evaluation of pig herds within the region that had previous outbreaks of ASF in the early 1990's. A total of 84 herds was initially selected based on data from previous outbreaks. These farms were visited by representatives of the Veterinary Services, who did an epidemiological inquiry and an inspection of the premises. Based on the

#### AFRICAN SWINE FEVER STATUS OF PORTUGAL

results of these visits, 32 herds were selected for attempts at tick collection. Traps were placed on all these premises, and ticks were collected on 13 of these. More than 45,700 ticks were collected, with varying numbers from the different premises. Ticks were tested for ASF virus as described above. At the time of the site visit, two of these premises had ticks that were positive for ASF virus: the index herd and a contact herd in the surveillance zone which had already been depopulated. Stone buildings are an important habitat for ticks, and any stone buildings on the premises where virus was isolated from ticks were destroyed and the owners were compensated.

Various experiments are ongoing at the veterinary college concerning ticks and their role in the transmission of disease. Results of these experiments suggest that the virus levels obtained from the ticks in this outbreak were too low to cause infection in pigs.

At the time of the site visit, repopulation was allowed in the infected area on a case-by-case basis. Producers must apply to repopulate. They must have a registration number and must adhere to certain rules such as no commingling on common grazing areas. No further evidence of disease has been found, and all movement restrictions have been removed from Community legislation.

## The extent of an active disease control program, if any, if the agent is known to exist in the region

The measures taken to control the outbreak in November 1999 were described in the previous paragraphs.

### The vaccination status of the region

No vaccination has been used.

#### Disease status of adjacent regions

There is no ASF in adjacent regions. Within the European Union, ASF only exists on the island of Sardinia.

## The degree to which the region is separated from regions of higher risk through physical or other barriers

ASF is currently known to exist only in sub-Saharan Africa and on the island of Sardinia. There is a significant water barrier between Portugal and Africa.

## The extent to which movement of animals and animal products is controlled from regions of higher risk, and the level of biosecurity regarding such movements

The movement of animals and animal products from non-EU countries is regulated according to Community legislation. This prohibits the entry of live susceptible animals and risky products (e.g., fresh pork) from regions of higher risk such as Africa.

### Livestock demographics and marketing practices in the region

The local office visited by APHIS during the site visit was one of five in Alentejo and services a sub-region of Alentejo. In the sub-region there are 600,000 small ruminants, 90,000 bovines, and 20,000 reproducing pigs. There are 250 large pig farms with greater than 100 sows. In all of Alentejo there are 60,000 reproducing pigs, 50,000 on intensive (confinement) operations and 10,000 on extensive (outdoor housing). There are 350 extensive farms producing the traditional Alentejo ham/pork. Extensively produced Alentejo pork goes mostly for local consumption. In extensive production, the pigs graze for one year or more. Many grazing pigs go to Spain for slaughter and Iberian ham production. In 1999, 22,000 pigs went from Alentejo to Spain from 50 farms. The other small farms slaughter for local consumption. In addition to this movement from Portugal to Spain, the converse also occurs. Pigs from Spain move to graze on grass and acorns and then return to Spain for slaughter. This exchange from Spain to Portugal and back is confined to certain grazing areas and the pigs must be certified for movement. This generally occurs from October to February, as this is the period of grazing and oak production.

Intensive production farms produce pork products and one-month old pigs for roast pig production. This production includes only crossbreeds of Alentejo and Duroc or other breeds. The intensive farms export only to other regions in Portugal.

# Policies and infrastructure for animal disease control in the region - i.e., emergency response capability

ASF is a compulsorily notifiable disease in Portugal, and a stamping out policy is enforced in the event of an outbreak. Control measures as laid down in both Community and national legislation are the basis of emergency measures, with additional surveillance and controls as necessary. Experience and control measures from previous eradication efforts have proved useful. The Ministry has the authority, policies, and resources to rapidly detect and effectively respond to an outbreak of ASF. This was demonstrated by the response to the outbreak of ASF in 1999, with rapid control and ongoing surveillance programs to detect any further outbreaks of the disease.

#### AFRICAN SWINE FEVER STATUS OF PORTUGAL

#### **Conclusions**

Based on the above, APHIS has reached the following conclusions:

- 1) ASF is not known to exist in Portugal at the present time. Extensive surveillance following the November 1999 outbreak demonstrated that the outbreak had been contained and eradicated.
- 2) The possible persistence of ASF virus in ticks was identified as the major risk factor by APHIS. ASF virus was isolated from ticks on two infected premises, but tick surveillance elsewhere in the region failed to demonstrate the existence of a tick reservoir of ASF virus.
- 3) Portugal maintains control over the movement of animals and animal products from regions of higher risk.
- 4) Portugal maintains a surveillance system capable of rapidly detecting any future occurrence of ASF.
- 5) Portugal has the laws, policies and infrastructure to detect, respond to, and eliminate any occurrence of ASF.

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